THE EFFECT OF 3-INDOLEACETIC ACID ON THE RESPONSE OF LACTOBACILLUS ARABINOSUS 17-5 TO NICOTINAMIDE

By ARTHUR W. GALSTON and MARGERY E. HAND

(From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena)

(Received for publication, November 15, 1948)

Lactobacillus arabinosus 17-5 has been widely used as an assay organism for nicotinic acid (NA) since the development of the method by Snell and Wright (1). Although it has been realized that other substances present in tissue extracts may interfere with the bioassay, the nature of such substances has not been elucidated.

During an investigation of tryptophan metabolism in the pea plant, we studied the possible conversion of this compound to nicotinic acid, since such a transformation has been demonstrated to occur in numerous organisms (2–4). The method involved infiltration of tissue with large quantities of tryptophan and subsequent bioassay for nicotinic acid with *Lactobacillus arabinosus* 17-5. Certain anomalous results led us to believe that other metabolites of tryptophan were interfering with the assay. Because 3-indoleacetic acid (IAA) is a known plant metabolite of tryptophan (5), we tested it for possible interference with the assay, and, as described below, found that such interference may occur under certain circumstances.

EXPERIMENTAL

The methods used were essentially those described by Snell and Wright (1). Stocks of *Lactobacillus arabinosus* 17-5 were carried as stab cultures in tubes containing the basal medium for the nicotinic acid assay plus 1 per cent agar fortified with 1 γ of nicotinamide per tube. These were incubated for 24 hours at 30° and were then removed to a refrigerator where they were stored until use. New stab cultures were prepared every 1 to 2 weeks. Organisms to be used in growth tests were transferred to liquid cultures containing basal medium plus 0.1 γ of nicotinamide per tube, and were incubated at 30° for 18 to 24 hours immediately prior to use. 1 drop of such a culture was used as the inoculum in the growth test, since it was found that the centrifugation of the culture and resuspension in saline recommended by Snell and Wright gave blanks which were not significantly smaller than ours.

Standard series were prepared containing 0, 0.05, 0.10, 0.15, 0.20, 0.30, 0.40, 0.50, and 1.0 γ of nicotinamide per tube. The experimental series contained various concentrations of IAA in addition to nicotinamide. Because of reports of differences among various lots of IAA (6), samples of this

product prepared by three different manufacturers were used. All IAA solutions were adjusted to pH 7.0. Assay tubes were incubated for 72 hours at 30°; 5 ml. aliquots were then pipetted into Erlenmeyer flasks, 3 drops of brom-thymol blue added, and the titration performed with 0.1 N NaOH.

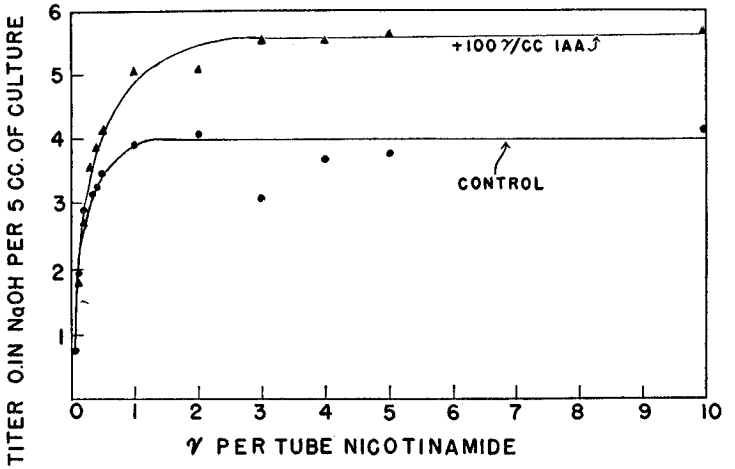

FIG. 1. The effect of 100 γ per cc. of IAA on the response of *Lactobacillus* to various concentrations of nicotinamide.

TABLE I

Comparative Effects of Indoleacetic, α-Naphthaleneacetic, and 2,4-Dichlorophenoxyacetic Acids on Response of Lactobacillus to Nicotinamide

Nicotinamide per tube	Titer of 0.1 N NaOH per 5 cc. culture medium			
	Control	+ 100 γ per cc. naphthaleneacetic acid	+ 100 γ per cc. 2,4-dichlorophenoxyacetic acid	+ 100 γ per cc. indoleacetic acid
γ	cc.	cc.	cc.	cc.
0	1.06	1.24	1.10	1.17
0.10	2.25	2.28	1.13	2.13
0.30	3.81	2.81	3.48	3.51
1.0	4.20	3.86	4.35	5.35
3.0	4.64	4.57	4.96	5.65
10.0	4.35	4.18	4.58	5.20

Whereas IAA is without effect on the growth of *Lactobacillus* in the absence of NA, it enhances the growth of the organism at the higher NA values of the standard series. A concentration of 40 γ per cc. of IAA was barely sufficient to produce a discernible effect, whereas 100 γ per cc. yielded a considerable growth effect. This concentration was therefore adopted as stand-

ard in these experiments. In order to determine whether this IAA effect would be magnified at still higher NA concentrations, new standard series were prepared, in which the range of NA additions was carried up as high as 100 γ per tube. As may be seen from Fig. 1, the stimulatory effect of IAA is maintained and even magnified at the higher levels of NA.

In higher plants, the growth-promoting effects of IAA may be duplicated by many aromatic organic acids (7). To see whether such materials could similarly substitute for IAA in the *Lactobacillus* growth effect, α-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid were applied in series parallel to the control standard series and the IAA-treated series. As is seen from Table I, they not only failed to stimulate significantly the growth of *Lactobacillus*, but actually depressed it at certain NA levels. The synergistic effect with NA seems, therefore, to be fairly specific for IAA.

DISCUSSION

The IAA effect described in this paper is probably of no significance in the *Lactobacillus* determination of NA because (*a*) the quantities of IAA present in normal tissues are insufficient to cause this effect and (*b*) the range of NA concentrations over which the IAA effect is exerted is not generally employed in assays. However, if tissues are infiltrated with tryptophan in order to study its conversion to nicotinic acid, the IAA formed enzymatically from the tryptophan may interfere with a *Lactobacillus* bioassay. In addition, since alkaline treatment of plant proteins may release bound IAA and may also convert tryptophan to IAA (8), such treatment should be avoided in tissue to be assayed for nicotinic acid. Kodicek (9) has reported that alkaline hydrolysis of grains yielded a material biologically inactive as NA which reacted with the cyanogen-*p*-aminoacetophenone reagent to give an intensified color. This interference with a *chemical* determination of NA may also be attributable to IAA.

The fact that a NA-IAA interaction exists in *Lactobacillus* as well as in higher plants (10) indicates some definite metabolic connection between these two physiologically important compounds. At present, we are unable to explain the nature of the interaction.

SUMMARY

1. Indoleacetic acid, itself without effect on the growth of *Lactobacillus*, enhances the growth of this organism in the presence of high concentrations of nicotinamide.

2. α-Naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid, which may replace indoleacetic acid in growth effects in higher plants, are without such effect on *Lactobacillus*.

3. Because of the large quantities of IAA needed to produce this effect,

it is unlikely that IAA normally interferes with the microbiological assay for nicotinic acid.

BIBLIOGRAPHY

1. Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, **139**, 675 (1941).
2. Beadle, G. W., Mitchell, H. K., and Nyc, J. F., *Proc. Nat. Acad. Sc.*, **33**, 155 (1947).
3. Krehl, W. A., Sarma, P. S., Tepley, L. J., and Elvehjem, C. A., *Science*, **101**, 489 (1945).
4. Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **163**, 343 (1946).
5. Wildman, S. G., Ferri, M. G., and Bonner, J., *Arch. Biochem.*, **13**, 131 (1947).
6. Cohen, S. S., and Fowler, C. B., *J. Biol. Chem.*, **168**, 775 (1947).
7. Went, F. W., and Thimann, K. V., Phytohormones, New York (1937).
8. Gordon, S. A., and Wildman, S. G., *J. Biol. Chem.*, **147**, 389 (1943).
9. Kodicek, E., *Biochem. J.*, **34**, 712 (1940).
10. Galston, A. W., *J. Biol. Chem.*, **169**, 465 (1947).